

Claims

- 5 1. A method for the cytosine methylation detection in a DNA sample, comprising the following steps:
- a) a genomic DNA sample is treated in a manner capable of distinguishing methylated from unmethylated cytosine bases;
- 10 b) the pre-treated DNA is amplified using at least one oligonucleotide primer, a polymerase and a set of nucleotides of which at least one is marked with a first type of label;
- 15 c) a sequence-specific oligonucleotide or oligomer probe is hybridized to the amplification product and a FRET occurs if the oligonucleotide or oligomer probe, marked with a second type of label, binds in close proximity to one of the labeled nucleotides that was incorporated into the amplification product;
- 20 d) the level of methylation of the sample is determined by the level of interaction between said first and second type of label.
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- 30 2. A method according to claim 1, characterised in that the first type of label is a donor fluorophore and the second type of label is an acceptor fluorophore and that the extent of fluorescence resonance energy transfer (FRET) is measured.
- 35 3. A method according to claim 1, characterised in that the first type of label is an acceptor fluorophore and the second type of label is a donor fluorophore

and that the extent of fluorescence resonance energy transfer (FRET) is measured.

- 5 4. A method according to claim 1, characterised in that the nucleotides of step b) contain a fluorescent moiety and the probe in step c) a quencher moiety.
- 10 5. A method according to claim 1, characterised in that the nucleotides of step b) contain a quencher moiety and the probe in step c) a fluorescent moiety.
- 15 6. A method according to claim 1, characterised in that the polymerase has no 5' to 3' exonuclease activity in order to prevent degradation of the probe.
- 20 7. A method according to claim 1, characterized in that a change in fluorescence intensity is monitored in real-time during the amplification reaction.
- 25 8. A method according to claim 1, characterized in that a change in fluorescence intensity is monitored at end-point of target amplification.
- 30 9. A method according to one of the preceding claims, characterized in that the amplification reaction is achieved with the polymerase chain reaction (PCR).
- 35 10. A method according to one of the preceding claims, characterized in that the probe contains only one CpG.
11. A method according to one of the preceding claims, characterized in that the probe contains several CpGs.

12. A method according to claim 11, characterized in that each probe for each CpG has a fluorescent label.
- 5 13. A method according to one of the preceding claims, characterized in that the probe can be end labeled or internally labeled.
- 10 14. A method according to one of the preceding claims, characterized in that the methylation information is determined by the change in fluorescence intensity during subsequent rounds of PCR.
- 15 15. A method according to one of the preceding claims, characterized in that the sample DNA is only amplified by chosen PCR primers if a certain methylation state is present at a specific site in the sample DNA.
- 20 16. A method according to one of the preceding claims, characterized in that the sample DNA is only amplified if a certain methylation state was present at a specific site in the sample DNA, the sequence context of which is essentially complementary to one or more oligonucleotides or PNA oligomers which are additionally used in the PCR reaction.
- 25 17. A method according to one of the preceding claims, characterized in that the amplification from the 3'-end of the probe is blocked by phosphorylation.
- 30 18. A method according to one of the preceding claims that a melting curve is generated at the end of the PCR to gather additional data.

19. A method according to one of the preceding claims wherein the fluorescent moiety is a fluorescein dye, a rhodamine dye, or a cyanine dye.
- 5 20. A method according to one of the preceding claims wherein the quencher moiety is a rhodamine dye.
21. A method according to one of the preceding claims wherein the deamination treatment of the DNA is performed with a bisulfite reagent.
- 10 22. A method according to one of the preceding claims whereby the DNA sample is cleaved prior to deamination treatment with restriction endonucleases.
- 15 23. A method according to one of the preceding claims whereby the DNA sample is isolated from mammalian sources e.g. cell lines, blood, sputum, faeces, urine, cerebrospinal fluid, tissue embedded in paraffin, for example, ocular tissue, intestine, kidney, brain, heart, prostate, lung, chest or liver, histological slides and all possible combinations.
- 20 24. Use of a pre-treated genomic DNA according to one of the preceding claims for the determination of the methylation status of a corresponding genomic DNA.
- 25 25. A diagnostic kit for the detection of the methylation of cytosine bases in genomic DNA samples, comprising reagents for the selective deamination of cytosine bases in genomic DNA, one or more primers and labeled nucleotides for the
- 30 amplification step, a detectable probe and
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optionally protocols or instructions for one of the methods according to one of the preceding claims.

1. A method for determining a concentration of a substance in a sample, comprising:
a) providing a sample;
b) measuring a signal from the sample;
c) determining a concentration of the substance in the sample based on the signal;
d) providing a reference sample;
e) measuring a signal from the reference sample;
f) determining a concentration of the substance in the reference sample based on the signal;
g) comparing the concentration of the substance in the sample to the concentration of the substance in the reference sample;
h) determining a concentration of the substance in the sample based on the comparison.